

fying indicia are not required to be located in the same field of view as shown in FIGS. 20 and 21.

#### Example 6

##### Plasma Treatment of Surfaces in the Cartridge

**[0129]** One useful technique for certain applications involves plasma treatment to clean a surface by ion bombardment and physical ablation of contaminants, particularly for elimination of organic contaminants. In addition, plasma treatment can be used to modify surfaces for attachment or adsorption of functional groups, such as for the printing of reaction sites. Furthermore, plasma treatment of a surface may modify the flow behavior of fluids coming into contact with that surface.

**[0130]** In one embodiment, a waveguide may be prepared for attachment of desired reaction sites by use of an argon/oxygen plasma cleaning. Following cleaning, epoxy-silane may be deposited on the cleaned surface of the waveguide to functionalize and prepare the waveguide for further processing.

**[0131]** Additionally, capillary flow within the cartridge may be enhanced by suitable coatings or treatments of both the flow plate and the waveguide. In one embodiment, both the flow plate and the waveguide are subjected to a plasma cleaning step. When assembled, capillary flow rate is greatly enhanced relative to cartridges having a flow plate that has not been subjected to a plasma treatment. Consequently, smaller volumes of sample (e.g., 50 microliters or less) may be needed to perform the analyte detection process.

#### Example 7

##### Disposable Assay Cartridge and Assay System

**[0132]** FIG. 26 shows a schematic representation of an exemplary disposable assay cartridge and a multiplexed fluorescence assay, in accordance with an embodiment. A protein microarray is printed to a plastic planar waveguide which is bonded to a plastic upper component to define a flow channel. Fluids, such as sample, wash, and detect reagents may be introduced via an inlet port. The assay surface may be illuminated by an evanescent field generated down the length of the multi-mode waveguide. The array is imaged in a single field of view through the plane of the waveguide.

**[0133]** At the TIR interface, an evanescent field is generated that decays exponentially into the aqueous medium. The decay length of the evanescent field is on the order of a hundred nanometers for visible light. For fluorescence assay applications, the advantage is localization of the illumination source precisely at the solid-liquid assay interface, limiting negative effects such as the bulk solution, line-of-sight, light scattering.

**[0134]** The cartridge is based on a thick (~1 mm), multi-mode planar waveguide fabricated by injection molding of a low auto-fluorescence plastic (e.g., cyclic olefin polymer). One of the major advantages of this cartridge configuration is the incorporation of a coupling lens into the molded waveguide (FIG. 27). This lens design overcomes fundamental challenge of reproducible light coupling to the waveguide in prior designs. The lens design creates a diverging beam such that modes mix down the length of the waveguide, eventually creating a spatially uniform illumination field along the axial length of the waveguide.

**[0135]** The plastic waveguides are activated with a surface chemistry treatment to render them amine-reactive. Details of the surface activation are similar to methods described in the literature, with proprietary modifications and improvements.

**[0136]** A protein array is printed to the activated surface of the planar waveguide prior to assembly into the cartridge. Details of the array features and layout are provided below. The arrays may be printed with a commercial arrayer, such as Bio-Dot AD3200 robotic arrayer equipped with Bio-Jet print head dispensing 28 nanoliter droplets. Resulting reaction site diameters are approximately 0.5 mm, and the arrays are printed on a grid with 1.25 mm centers. The length of the 30 feature (i.e., 2 rows by 15 columns in the present example) array is approximately 17.5 mm. After printing, the waveguide arrays are rinsed with a protein-based blocking agent, spin-dried, and then coated with a sugar-based stabilizer for storage.

**[0137]** Printed waveguides are assembled into an injection molded cartridge to form a 2 to 5 mm-wide fluidic channel with a volume of approximately 30 microliters. The cartridge inlet port provides a reservoir for introduction of assay fluids. The exit port provides a fluidic contact to an absorbent pad that serves as a waste reservoir. The cartridge is configured to provide reproducible passive fluid flow, driven by a combination of capillary action and hydrostatic pressure, as described in, for instance, U.S. Provisional Patent Application Ser. No. 61/391,911 entitled "Fluidic Assay Cartridge with Controlled Passive Flow" filed 11 Oct. 2010. All fluids stay on board the cartridge upon completion of the assay procedure, thus minimizing biohazard. In this way, a combination of printed antigens, controls, and a sample placed in the liquid channel may be used to perform an assay, as schematically shown in FIG. 27.

**[0138]** Returning to FIG. 26, further details of the cartridge and assay system are described. FIG. 26 illustrates a cross-sectional view of an assay system 2600, including a cartridge 2602. Cartridge 2602 includes a planar waveguide 2605 with an integrated lens 2610 suitable for use with the labeled antigen assay of FIG. 27, in accordance with the embodiment (See also U.S. patent application Ser. No. 12/617,535, which is incorporated herein in its entirety by reference). An illumination beam 2615 is inserted into planar waveguide 2605 through integrated lens 2610. Illumination beam 2615 may be provided, for example, by a laser with an appropriate wavelength to excite fluorescent labels at an assay surface 2620. Other appropriate forms of illumination, either collimated or uncollimated, may also be used with assay system 2600. Integrated lens 2610 is configured to cooperate with planar waveguide 2605 such that illumination beam 2615, so inserted, is guided through planar waveguide 2605 and may illuminate assay surface 2620 by evanescent light coupling. Assay surface 2620, an upper component 2628, which includes an inlet port 2630 and an output port 2635, cooperate to define a fluidic sample chamber 2640. Assay surface 2620 and upper element 2628 can be bonded via a channel-defining adhesive gasket 2625 or via direct bonding methods such as laser welding, ultrasonic welding, or solvent bonding. Appropriate chemical compounds (such as a printed antigen) are bound to assay surface 2620 such that when a biological sample and labeled detect reagent are added to the fluidic sample chamber 2640, a target analyte, if present, forms a sandwich between its specific labeled detect reagent and its specific chemical compound immobilized on assay surface 2620. If the specific complex is formed at assay surface 2620,